(54) Title: BIOFUNCTIONAL HYDROXYAPATITE COATINGS AND MICROSPHERES FOR IN-SITU DRUG ENCAPSULATION
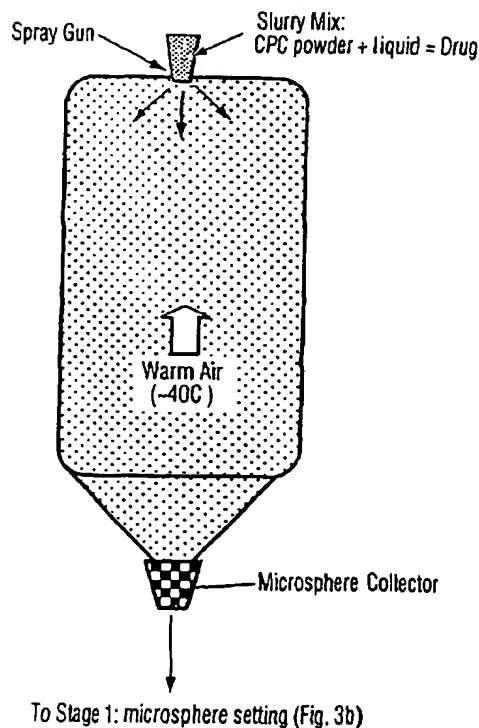
(57) Abstract: This invention relates to novel room-temperature process for obtaining calcium phosphate, in particular hydroxyapatite, coatings and microspheres that encapsulate drugs, proteins, genes, DNA for therapeutical use. The coatings and microspheres are designed to perform a defined biological function related to drug delivery, such as gene therapy through gene delivery. A novel method for encapsulation, and subsequent controlled release of therapeutically active agents from such biofunctional coatings and microspheres is disclosed. Such coatings and microspheres are useful for side-effects free, long-term, targeted, controlled release and delivery of drugs, proteins, DNA, and other therapeutic agents.

Spray Gun — Slurry Mix: CPC powder + liquid = Drug

Warm Air (~40C)

Microsphere Collector

To Stage 1: microsphere setting (Fig. 3b)

WO 02/085330 A1

European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BIOFUNCTIONAL HYDROXYAPATITE COATINGS AND MICROSPHERES FOR IN-SITU DRUG ENCAPSULATION

TECHNICAL FIELD

5

This invention relates to novel room-temperature process for obtaining calcium phosphate, in particular hydroxyapatite, microspheres and coatings with encapsulated drugs, proteins, genes, DNA for therapeutical use. The coatings and microspheres are designed to perform a defined biological function related to drug

10   delivery, such as gene therapy through gene delivery. A novel method for encapsulation, and subsequent controlled release of therapeutically active agents from such biofunctional coatings and microspheres is disclosed. Such coatings and microspheres are useful for side effects - free, long-term, targeted, controlled release and delivery of drugs, proteins, DNA, and other therapeutic agents.

15

BACKGROUND OF THE INVENTION

Rapid progress in the human genome project promises that diseases that could not be treated before can be curable in near future. The expectation is that the trial-

20   and-error era of fighting illnesses by addressing the symptoms is coming to an end. Consequently, the issue of drug and gene release control becomes increasingly critical. Currently, many types of new drugs, or genes, have to be administered by daily injections, or even several times per day. An entirely new approach to drug delivery is therefore necessary to fully utilize the advantages of new drugs resulting from the

25   genome project. Slow but steady release of drugs is sought in treatment of many diseases, from cancer and Parkinson's disease, to hormonal treatment of obesity where directly administered hormones reside in human body only for short period of time. In the past, polymeric materials have been used for drug delivery control and enjoyed substantial clinical success for certain drug systems. The need for alternative inor-

30   ganic drug delivery systems, offering more flexibility in drug-carrier system selection, bioresorption and release control, hydrophobic / hydrophilic property control, and negligible side effects, is just emerging. Hydroxyapatite (HA) matrix for drug encapsulation, being already the principal inorganic component of bone, offers entirely new perspectives for drug delivery systems.

35

Hydroxyapatite Ceramics, $Ca_{10}(PO_4)_6(OH)_2$, belong to a large class of calcium phosphate (CaP) based bioactive materials used for a variety of biomedical applications, including matrices for drug release control [M. Itokazu et al Biomaterials, 19,817-819,1998; F. Minguez et al Drugs Exp. Clin. Res., 16[5], 231-

- 2 -

235,1990; W. Paul and C. P. Sharma, J. Mater. Sci. Mater. Med., 10, 383-388,1999].
Other members of the CaP family, such as dicalcium phosphate $CaHPO_4·2H_2O$ or
tricalcium phosphate $Ca_3(PO_4)_2$, have also been used for similar purposes. The CaP
family of materials has been long recognized as having highest degree of
5   biocompatibility with human tissue.

        Calcium Phosphate Cements (CPC) were reported in a binary system
containing tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrate
(DCPA) [L.C. Chow et al. J. Dent Res., 63, 200,1984]. The CPC advantages of self-
10   setting and apatitic phase, e.g. HA, as an end product led to applications such as bone
replacements and reconstruction, and also drug delivery [M.Dairra, et al. Biomaterials,
19 1523-1527, 1998; M. Otsuka, et al. J. of Controlled Release 43(1997)115-122,
1997; Y. Tabata, PSTT, Vol.3, No.3, 80-89, 2000; M. Otsuka, et al. J. of Pharm. Sci.
Vol.83, No.5, 1994]. CPC is typically formulated as a mixture of solid and liquid
15   components in pertinent proportions, which react to form the apatite. The
physicochemical reactions that occur upon mixing of the solid and liquid components
are complex, but dissolution and precipitation are the primarily mechanisms responsi-
ble for the final apatite formation [C. Hamanish et al J. Biomed. Mat. Res., Vol.32,
383-389,1996; E. Ferandez et al J. Mater. Sci.Med.10,223-230, 1999].   The
20   reaction pathway in most CPC systems does not lead to stoichiometric HA, but rather
calcium-deficient $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, similar to that found in bone. The
process parameters, such as Ca/P ratio, powder/liquid ratio, seeds concentration and
type, nature of reagents, control the final properties, such as phase content, porosity,
strength, setting time, phase transformation kinetics, and microstructure of CPC-
25   derived hydroxyapatite (CPC-HA). Bermudez et al [J. Mat. Sci. Med. 4, 503-
508,1993; ibid 5, 67-71, 1994] correlated the compressive strength of CPC vs starting
Ca/P ratio in the systems of monocalcium phosphate monohydrate (MCPM) and
calcium oxide. The optimum Ca/P ratio was found in a range of 1.25 - 1.45. Brown et
al. [J. Am. Cerm. Soc. 74[5] 934-40,1991] found that the kinetics of HA formation at
30   low temperatures in DCP / TTCP system are initially controlled by the surface area of
the reactants, and eventually by diffusion. These process variables will be used in the
present project to control crystallinity, and thus resorption and drug release rate from
the HA microspheres.

35   **Biomimetic Deposition** of HA films at room temperature (BM-HA) was used
for a variety of biomedical applications, including drug delivery [H. B. Wen et al, J.
Biomed. Mater. Res., 41, 227-36,1998; S. Lin and A. A. Campbell, US Pat 5958430,

- 3 -

1999; D. M. Liu et al J. Mater. Sci. Mater. Med., 5, 147-153,1994; K. de Groot et al, J. Biomed. Mater. Res., 21, 1375-1381,1987]. This forming mechanism is driven by supersaturation of Ca^{2+} and PO_4^{3-}, under appropriate solution pH, where HA is the most stable phase. The apatitic crystals form through nucleation and growth, and may

5   incorporate biologically active species, such as antibiotics, anti-cancer drugs, anti-inflammatory agents, etc. The deposition rates are however small for BM-HA, generally in the range of 0.05-0.5μm/h, and high concentration dosage of drug is difficult to achieve. Therefore, stand-alone BM-HA is not suitable if the goal is to form films in excess of 1μm, but may be appropriate as an additional encapsulating

10   film, on top of the porous HA structure saturated with the drug material. This is especially critical for orthopedics where high concentration of antibiotics is required at bone-HA interface to prevent acute inflammation in early after-operation stages. Furthermore, the physiological solutions for BM-HA formation are naturally water-based, which makes impossible to encapsulate hydrophobic bioactive agents into BM-

15   HA coatings.

Sol-Gel Deposition of HA (SG-HA) films at elevated temperatures (375-500C) was disclosed previously by D. Liu and T. Troczynski in U.S. patent application Serial No. 09/563,231, filed May 2, 2000, the subject matter of which is incorpo-

20   rated herein by reference. Sol-gel (SG) processing of HA allows molecular-level mixing of the calcium and phosphor precursors, which improves chemical homogeneity of the resulting calcium phosphate. The versatility of the SG method opens an opportunity to form thin film coatings in a simple, mild, relatively low-temperature process. The crystallinity of the calcium phosphate phase obtained through the novel

25   inventive process by D. Liu and T. Troczynski can be enhanced by appropriate use of water treatment during processing. Variation of Ca/P ratio in the sol-gel precursor mix allows one to obtain other than calcium phosphate phases, for example, hydroxyapatite, dicalcium phosphate, tricalcium phosphate or tetracalcium phosphate. The use of SG-HA thin (<1 μm) dense highly crystalline films to nucleate and grow

30   thick (>10 μm) CPC-HA porous, low-crystallinity (amorphous) films for drug encapsulation and release is hereby disclosed.

Problems With Drug Delivery in Vivo are related to toxicity of the carrier agent, the generally low loading capacity for drugs as well as the aim to control drug

35   delivery resulting in self-regulated, timed release. With the exception of colloidal carrier systems, which support relatively high loading capacity for drugs, most systems deliver inadequate levels of bioactive drugs. In terms of gene delivery, to date

- 4 -

the most efficient, though least safe methods of delivery are through viral mediated
gene transfer. It is highly inefficient method, and is faced with even greater problems
than the delivery of drugs due to the hydrophilic and labile nature DNA oligos. The
problems with delivery of genes or antisense oligos originate from the rapid clearance
5   of plasmid DNA or oligos by hepatic and renal uptake as well as the degradation of
DNA by serum nucleases [Takura Y, et al. Eur. J. Pharm. Sci. 13 (2001) 71-76].
These effects have been observed for both in situ and intravenous delivery. For
example it was estimated that more than half of the intravenous or in situ delivered
naked plasmid DNA was cleared from the tumor site within the first two hours
10   following intratumoral injection [Ohkouchi, K., et al Cancer Res. 50, (1996)
1640-1644 and Imoto, H., et al. Cancer Res. 52, (1992)., 4396-4401]. Even after
clearance, only a small percentage of the remaining DNA or oligos make their way to
the cytoplasm or nucleus of the target cell. The membrane permeability of naked DNA
and especially oligos is virtually nonexistent, due to their polyanionic nature. For this
15   reason, their uptake through the endosomal compartment is associated with a severe
drop in pH and degradation. Finally, many of the genes delivered have to be trans-
ported and sometimes incorporated in the genome of the target cell for stable expres-
sion. This makes very difficult gene transfer in vivo. In addition, successful con-
trolled release is still problematic as for most applications (with the exception of
20   naked DNA vaccines) it is desirable to have a prolonged expression of the gene of
interest to ameliorate a particular medical condition. In most applications anywhere
from a few weeks to several months are desired for the expression of a certain gene
product.

25   **Drug Encapsulation in HA** has been achieved in the past by simple post-
impregnation of a sintered, porous HA ceramic [K. Yamamura et al, J. Biomed.
Mater. Res., 26, 1053-64,1992]. In this process, the drug molecules simply adsorb
onto surface of the porous ceramic. The drug release is accomplished through
desorption and leaching and the drug to the surrounding tissue after exposure to
30   physiological fluid. Unfortunately, most of the adsorbed drug molecules release from
such system in a relatively short period of time. Impregnation of drug material into
porous sintered calcium phosphate microspheres has been reported in patent literature.
"Slow release" porous granules are claimed in US Pat. 5,055,307 [S. Tsuru et al,
1991], wherein the granule is sintered at 200-1400C and the drug component impreg-
35   nated into its porosity. "Calcium phosphate microcarriers and microspheres" are
claimed in WO 98/43558 by B. Starling et al [1998], wherein hollow microspheres are
sintered and impregnated with drugs for slow release. D. Lee et al claim poorly

crystalline apatite [WO98/16268] wherein macro-shapes harden and may simulta-
neously encapsulate drug material for slow release. It has been suggested to use
porous, composite HA as a carrier for gentamicin sulfate (GS), an aminoglycoside
antibiotic to treat bacterial infections at infected osseous sites [J. M. Rogers-Foy et al,

5   J. Inv. Surgery 12 (1997) 263 – 275]. The presence of proteins in HA coatings did not
affect the dissolution properties of either calcium or phosphorus ions and that it was
solely dependent on the media [Bender S. A. et al. Biomaterials 21 (2000) 299-305].

The group of Kobe University lead by Prof. M. Otsuka performed series of
10   investigations of drug encapsulation in self-setting calcium phosphate cements
derived from tetracalcium phosphate and dicalcium phosphate [J. Contr. Rel. 43 115-
122 1997; ibid 52 281-289 1998; J. Pharm. Sci. 83 611-615, 259-263, 255-258,
1994]. The cement was shaped with in-situ drug encapsulation, into 15mm diameter
macro-pellets and drug (indomethacin) release monitored over up to 3 weeks period.
15   It was concluded that the cement-drug delivery system, shaped in-situ into surround-
ing bone tissue, may be an excellent way to treat localized bone infections with high
therapeutic effectiveness. The advantage of HA for drug delivery is that side effects
have never been a concern for hydroxyapatite materials [Y. Shinto et al, J. Bone Jt.
Surg., 74B4, 600-4,1992]. Calcium phosphate – biodegradable polymer blends were
20   also investigated as possible vehicles for drug delivery [I. Soriano and C. Evora, J.
Contr. Rel. 68 121-134 2000]. Prolonged drug release (up to 10 weeks) was obtained
for the composites coated with hydrophobic polymer coatings. A group from Univer-
sity of Pennsylvania [Q. Qiu et al J. Biomed Mat Res. 52 66-76 2000] recently
processed polymer-bioactive glass-ceramic composite microspheres for drug delivery.
25   Porous calcium phosphate ceramics were impregnated with bone marrow cells [E.
Kon et al, J. Biomed Mat. Res. 49 328-337 2000] and with human bone
morphogenetic protein [I. Alam et al J. Biomed Mat. Res. 52 2000].

S. Takenori et al., in U.S. Patent No. 5,993,535 (and accompanying
30   EP0899247), disclosed a calcium phosphate cement comprising tetracalcium phos-
phate and calcium hydrogen phosphate polysaccharide as main components. It needed
24hours incubation at 37°C for conversion of hydroxyapatite. T. Sumiaki et al., in
U.S. Patent No. 5,055,307, disclosed slow release drug delivery granules comprising
porous granules of a calcium phosphate compound having a ratio of Ca to P of 1.3 to
35   1.8, a porosity of 0.1 to 70%, a specific surface area of 0.1 to 50 m^2/g and a pore size
of 1nm to 10 μm. The granules were fired at a temperature of 200 to 1400°C, and a
drug component impregnated in pores of the granules, and a process for producing the

same. S. Gogolewski, in WO00/23123, disclosed the hardenable ceramic hydraulic cement comprising a drug to be delivered to the human or animal body upon degradation or dissolution of the hardened cement. However, conversion of CPC to achieve HA needed 40 hours. L. Chow et al., in U.S. Patent No. 5,525,148, disclosed calcium

5   phosphate cements, which self-harden substantially to hydroxyapatite at ambient temperature when in contact with an aqueous medium. More specifically the cements comprise a combination of calcium phosphates other than tetracalcium phosphate with an aqueous solution adjusted with a base to maintain a pH of about 12.5 or above and having sufficient dissolved phosphate salt to yield a solution mixture with phosphate

10   concentration equal to or greater than about 0.2 mol/L. However, major disadvantages of the processing are that high pH (>12.5), which could denature most of drugs, proteins and DNA, so the process is not suitable for drug encapsulation vehicles. C. Rey et al., in WO9816209, disclosed a synthetic, poorly crystalline apatite calcium phosphate containing a biologically active agent and/or cells, preferably tissue-

15   forming or tissue-degrading cells, useful for a variety of in vivo and in vitro applications, including drug delivery, tissue growth and osseous augmentation. However, the ratio of Ca/P was limited less than 1.5, and the authors did not disclose how to fabricate the microspheres and coatings.

20   The physical characteristics, i.e. shape, of the hydroxyapatite ceramic, also have significant impact on the tissue response. Among different possible shapes, hydroxyapatite granules facilitate not only surgical operations but also benefit the tissue growth after implantation by creating relatively large inter-granular pores allowing invasion by the host tissue. On this basis, the use of drug-containing HA

25   granules has enhanced therapeutic effect in practical clinical/biomedical applications.

While the above studies describe the dissolution of porous HA to release a soluble extracellular acting bioactive ingredient through desorption and leaching, this disclosure aims also intracellular delivery of genes, drugs or proteins using resorbing

30   HA microcarriers. The key observation is that HA particles were found inside macrophages at the interface of HA-coated hip implants [Bauer T. W. et al. J. Bone Joint Surg. Am. 73-A (1991) 1439-1452]. Consequently it was proposed that phagocytes take up and solubilize HA particles [Evans R. W. et al. Calcif. Tissue. Int. 36 (1984) 645-650]. This may be therefore one of the principal mechanisms of

35   intracellular delivery of genes through use of HA coatings and microspheres, without the need for use viral mediated gene transfer.

SUMMARY OF INVENTION

5

10

15

We hereby disclose a process through which hydroxyapatite can be engineered to function as an efficient vehicle for drug delivery from coatings or self-supported microspheres. The process relates to calcium phosphate (CaP), in particular hydroxyapatite (HA) microspheres and coatings capable to encapsulate any type of drug or protein which can be dispersed in organic liquids or water. The process starts with calcium phosphate cement (CPC) slurry, followed by incubation to precipitate HA phase within the microsphere or within the coating. By adding drugs and proteins into colloidal suspension (CPC slurry) of the microsphere or coating precursors, a direct, in-situ encapsulation, and subsequent controlled release of the therapeutically active agents from the apatite microspheres is achieved. The varying degree of crystallinity of the microspheres is used to control and customize their resorption process in body fluids, and thus the rate of drug release.

20

25

Despite the fact that several techniques were investigated in the past in use of static macro-components of CaP for drug delivery, none of them allows processing of fast setting functionally gradient cement microspheres and coatings with in-situ drug encapsulation. Consequently we disclose hereby a new, safe and inexpensive way to deliver drugs, proteins, genes and antisense oligos in vivo. In this process calcium phosphate coatings and microspheres are obtained through dissolution-precipitation mechanism similar to setting of calcium phosphate cements (CPC). This process is used for deposition of adhesive, thick (typically of 10-1000 µm thickness) HA films and for making HA microspheres (typically of 10-1000 µm diameter).

30

35

Calcium phosphate cements, like most other cements (e.g. Portland cement) release heat of hydration when hydrating and setting. When setting fast (which is an important requirement in certain applications), this heat may not dissipate fast enough and temperature of cemented area increases, sometimes to a level high enough to damage, e.g. crack, the cement. More importantly, if the cement is applied in human body, e.g. as part of an implant, this temperature increase may damage the surrounding biological constituents such as cells, proteins, and enzymes when the heat is rapidly dissipated upon setting. The novel cement system disclosed here, experiences a relatively mild temperature rise during setting, i.e. from room temperature, ~20°C, to a maximum near body temperature, ~37°C. This cement also exhibits excellent mechanical properties. For example, its compressive strength is generally greater than 10 MPa, and in certain compositions, > 30 MPa.

- 8 -

This CPC slurry is a mixture of acidic calcium phosphates, such as monocalcium phosphate (MCP) anhydrate or dihydrate, and basic calcium hydroxide, together with a small amount of other inorganic ingredients, such as apatite seeds. This mixture is thoroughly mixed, e.g. using ball milling. The mixture, which is

5   suspended in an inert liquid medium (e.g. alcohols) or a liquid mixture of the inert medium and a small amount of phosphating liquid, has a starting Ca/P ratio in the range of 1.2 – 1.67 and a solid concentration of 30-70 vol%, before the shaping process is being carried out (i.e. filling the cavity with the cement or shaping a drug-impregnated microsphere). The shaping procedures can be optimized upon control of

10   the setting time within 2-40 minutes.

The resulting cement exhibits an apatitic phase (HA) with either an acicular grain or plate-like grain morphology, depending upon processing parameters. A nano-structured cement can also be synthesized and is used particularly for encapsulating

15   drugs, antibiotics, proteins, and enzymes. The apatite phase develops within 1 - 6 h of incubation within the CPC system disclosed. Fast formation of the apatite phase is advantageous for an early biological response with the surrounding host tissue. A fast setting (2-40 min) and hardening time (less than 6 hours) benefits enormously clinical applications of the cement. Controllable crystallinity, from amorphous (easily

20   resorbable) to highly crystalline (i.e. stable in physiological environment), of the final apatitic structure allows application-oriented customization of the cement. Fine (nano-to-submicron) starting ingredients allow final microstructure to be well controlled, allowing high strength of the final body. Nano-structure of the novel CPC offers great advantage for encapsulating drugs, as well as for their controlled release.

25   Apatite microspheres can be easily fabricated using the novel CPC formulation, with size ranging from 10-1000 microns, using simple spray-drying equipment. Control of the setting and hardening process of the cement microspheres allows a variety of biomedical or clinical applications, e.g., injection-packing, and in–situ hardening for repair, restoration, augmentation of defective tissues, and many others.

30

The disclosed process can be utilized to synthesize HA ceramics of different physical forms, at room temperatures. The following applications are targeted: granules or bulk shapes for artificial bone filler / bone reconstruction; room-tempera-ture processed coatings (on Ti and other alloys), with drug incorporation; or-

35   ganic/inorganic composites, including HA in combination with other materials such as biopolymers and ceramics, and proteins to form nano- and bio-composites with controlled drug release function and / or bone growth control functions. ·

We disclose here a novel approach to achieve a multi-layer, functionally gradient HA coatings or microspheres. That is, several layers of HA coatings are deposited using different techniques, with different functions assigned to any particular layer. In particular, we describe an *in-situ* drug encapsulation within the function-

5   ally gradient HA coatings processed by combining elevated-temperature sol-gel processing with room-temperature precipitation processing. As a result of this protocol, a 10-30 μm thick, nano-to-submicron porous HA matrix, firmly attached to a metallic substrate through a 0.6-0.8 μm thin intermediate HA film is achieved. The thin sol-gel HA film (SG-HA) develops good bond with the underlaying substrate at

10   the 400C processing temperature, and acts as an intermediate nucleation/bonding layer between the underlying substrate and the HA thick film matrix. The CPC-HA thick film matrix is deposited at room-temperature on the thin SG-HA film by dip coating in non-aqueous suspension of monocalcium phosphate and calcium hydroxide precursor powders. The drug material is dispersed in the suspension and initially

15   deposited within the pores of the precursor powder film. Subsequently, a porous thick film apatite is formed under physiological environment, through dissolution-precipitation process. The thin SG-HA film acts as a seeding template allowing nucleation and growth of the thick film apatite crystals, and therefore imparts sufficient interfacial strength to the HA matrix. This room-temperature coating process, associated with an

20   *in-situ* encapsulation of drugs, can be applied onto variety of metallic or non-metallic substrates, providing potential advantages in various biomedical/biochemical applications.

The microspheres are self-supporting granules produced through spray-drying

25   and incubation process of CPC-HA precursors slurry, using sythesis routes and chemistry much the same as for the coatings. Further details of the invention are provided below.

A room-temperature process for deposition of adhesive, thick hydroxyapatite

30   films on metallic substrates through dissolution-precipitation mechanism similar to setting of calcium phosphate cements (CPC) is disclosed. Such nanoporous, semi-amorphous 10-1000 μm thick film is rapidly grown on the underlying crystalline thin film of hydroxyapatite deposited through sol-gel process. A direct, *in-situ* encapsulation, and subsequent controlled release of therapeutically active agents from the

35   apatite coatings has been achieved. Slight modification of similar process leads to formation of medicated HA microspheres of 10-1000 μm diameter. Both the coatings

and microspheres are designated for side-effects free, long-term, targeted, controlled release and delivery of drugs, proteins, DNA, and others.

The invention is directed to a process for preparing a calcium phosphate phase which comprises: (a) dispersing a homogenization of monocalcium phosphate $Ca(H_2PO_4)_2$ and calcium hydroxide $Ca(OH)_2$ precursors in a substantially water-free medium to form a paste or viscous slurry of precursors; (b) drying the precursors slurry ; (c) admixing a water-based solution of phosphate ions in the slurry to produce a mixture; and (d) incubating the resulting mixture in a humid environment at a temperature of 20-50°C to dissolve the precursors and precipitate a calcium phosphate phase.

The calcium phosphate phase can be hydroxyapatite. The Ca/P atomic ratio in the precursors mixture can be in the range of 1.2 – 1.67 and the mixture can have a solids concentration of 30-70 vol%. The substantially water-free medium can be an alcohol. The alcohol can be ethyl alcohol, methyl alcohol or propyl alcohol The water-based solution of phosphate ions can be sodium phosphate and the humid environment can be 50% to 100% Relative Humidity.

The precursors slurry can be dried in a prescribed shape. The shape can be a pellet, a coating, a microsphere, or a dental cavity or bone cavity fill. A crystalline hydroxyapatite powder can be added to the precursors slurry to promote crystallization of hydroxyapatite calcium phosphate phase during the incubation period. A therapeutically active material can be added to the precursors slurry for encapsulation during crystallization of the hydroxyapatite calcium phosphate phase during the incubation period. The therapeutically active material can be a drug, a protein, a gene or DNA.

The slurry of precursors can be deposited and dried on the surface of a calcium phosphate substrate. The calcium phosphate substrate can be a thin film of hydroxyapatite coating deposited on a metallic substrate using sol-gel process. The precipitated calcium phosphate phase can be exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-} ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium phosphate phase.

The slurry of precursors can be atomized and spray-dried to obtain microspheres of the precursors. The microspheres of the precursors can be exposed to a water-based solution of phosphate ions and incubated in a humid environment at a

temperature of 20-50°C to promote dissolution of the precursors and precipitation of calcium phosphate phase. The microsphere containing the precipitated calcium phosphate phase can be exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-}ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium

5   phosphate phase. The precipitated calcium phosphate phase can be exposed to a solution polymer to form a polymer film on the microsphere surface.

BRIEF DESCRIPTION OF DRAWINGS

10   In drawings which illustrate specific embodiments of the invention, but which should not be construed as restricting the spirit or scope of the invention in any way:

Figure 1(a) illustrates a CPC-HA coating on a metallic substrate without SG-HA coating interlayer.

15

Figure 1(b) illustrates a CPC-HA coating on a metallic substrate with SG-HA coating.

Figure 2(a) illustrates a cross-sectional view of the CPC-HA/SG-HA coating
20   system.

Figure 2(b) illustrates a FTIR spectrum of the CPC-HA/SG-HA coating system.

25   Figure 3(a) illustrates a schematic of a spray-drying system for CPC microspheres for drug encapsulation.

Figure 3 illustrates the four stages of CPC-HA microsphere processing.

30   Figure 4(a) illustrates CPC-HA microspheres, ~20 µm large.

Figure 4(b) illustrates CPC-HA microspheres ~300 µm large.

Figures 5(a) and 5(b) illustrate respective CPC-HA microstructure: crystalline
35   (a) and amorphous (b).

Figures 6(a) and 6(b) illustrate biomimetic hydroxyapatite (BM-HA) film deposits, termed CPL, for calcium phosphate layer, on SG-HA substrate of low crystallinity (a) and of high crystallinity (b).

5   Figure 7 illustrates a plot of % Release vs. Time of Release (hours) for the HA-CPC coating with 5% of amethopterin drug encapsulated.

DETAILED DESCRIPTION OF INVENTION

10   In order to produce the thick HA films for drug encapsulation, surface of the underlying substrate (typically stainless steel or titanium) is first coated with thin, submicrometer film of sol-gel HA, as disclosed previously in U.S. patent application Serial No. 09/563,231, filed May 2, 2000, the subject matter of which is incorporated herein by reference. The thin sol-gel HA film (SG-HA) develops good bond with the

15   underlying substrate at the 400°C processing temperature, and acts as an intermediate nucleation and bonding layer between the underlying substrate and the CPC-HA thick film matrix. The CPC-HA thick film matrix is deposited at room-temperature on the thin SG-HA film by dip coating in non-aqueous suspension of monocalcium phosphate and calcium hydroxide precursor powders. The drug material is dispersed in the

20   suspension and initially deposited within the pores of the precursor powder film. Subsequently, the precursor powders film, containing drug material, is exposed to water-based solution of sodium phosphate and placed in an incubator at 37°C, 100% relative humidity, for up to 24 h. During this period, a porous thick HA film forms due to dissolution of the precursor powders and re-precipitation of apatite phase

25   encapsulating the drug material. The thin SG-HA film acts as a seeding template allowing nucleation and growth of the thick film apatite crystals, and therefore imparts sufficient interfacial strength to the HA matrix. As a result of this protocol, a 10-1000 μm thick, nano-to-submicron porous CPC-HA matrix encapsulating the drug material, firmly attached to a substrate through a 0.6-0.8 μm thin intermediate SG-HA film is

30   achieved. Figure 1 illustrates CPC-HA coating on metallic substrates (a) without SG-HA coating interlayer and (b) with SG-HA coating. Absence of the SG-HA coating interlayer causes spontaneous separation of CPC-HA coating from the substrate. Figure 2 shows cross-sectional view of the CPC-HA/SG-HA coating system (a) and its FTIR spectrum (b) compared with commercial crystalline hydroxyapatite. An

35   excellent, intimate interface between the two films of CPC-HA/SG-HA coating system confirms that SG-HA acts as a nucleation site for CPC-HA.

- 13 -

In order to produce the HA microspheres for drug encapsulation, the non-aqueous suspension of CPC precursor powders (monocalcium phosphate and calcium hydroxide), together with fine crystalline HA additive seeds, is atomized and spray dried to produce 10-1000 μm large approximately spherical particles (Figure 3a, and

5   Stage 1 in the schematic Figure 3). The drug material is dispersed in the suspension and initially deposited within the pores of the precursor powder microspheres. Subsequently, the precursor powders microspheres, containing drug material, are exposed to water-based solution of sodium phosphate and placed in an incubator at 37°C, 100% relative humidity, for up to 24 h (Stage 2). During this period, a porous

10   HA microsphere forms due to dissolution of the precursor powders and re-precipitation of apatite phase encapsulating the drug material, Stage 3. The fine crystalline HA additive particles act as seeds allowing nucleation and growth of the apatite crystals. As a result of this protocol, a 10-1000 μm large, nano-to-submicron porous CPC-HA microspheres matrix encapsulating the drug material is achieved. In the last Stage 4 of

15   the process (Fig. 3), a thin film of BM-HA may be deposited on the microsphere surface for additional encapsulation. Figure 4 is SEM micrograph of two CPC-HA microspheres, ~20 um large (a) and ~300 um large (b), produced according to the above process (BM-HA is not present on these microspheres). By changing the content of HA seeds in the microspheres, crystallinity (and thus resorption rate in

20   physiological environment) of the resulting CPC-HA may be varied. This is illustrated in Figure 5, showing microcrystalline (a) and amorphous (b) structure of CPC-HA (these are confirmed by XRD and FTIR data).

The coatings and microspheres are processed such as to encapsulate secondary

25   materials within the open and closed porosity of HA, the secondary material being preferably for therapeutical use, such as drugs, proteins, genes, DNA and the like. We have demonstrated that a direct, *in-situ* encapsulation, and subsequent controlled release of therapeutically active agents from such apatite coatings and microspheres can be achieved. Both the coatings and microspheres are designated for side-effects

30   free, long-term, targeted, controlled release and delivery of drugs, proteins, DNA, and others. The disclosed process addresses the issue of drug delivery through encapsulation (entrapment within the structure) rather than impregnation of drugs into HA matrix. Thus, the drug release is linked to resorption of the HA matrix, rather than leaching from the matrix. Materials process engineering has been developed to

35   control structure of HA for drug encapsulation, and therefore to control the HA resorption and drug or gene delivery process. The bio-resorbable HA for drugs encapsulation is designed and processed to release the drug as a result of gradual

resorption of the matrix, rather than leaching of the drug from open porosity of the matrix. The additional BM-HA encapsulation film grown on SG-HA coated substrate is shown in SEM micrographs in Figure 6. The biomimetic hydroxyapatite (BM-HA) film deposits, termed CPL here for calcium phosphate layer, are shown on SG-HA

5   substrate of low crystallinity (a) and high crystallinity (b).

In the specification and the claims, it is understood that when appropriate, the term "calcium phosphate" (CaP) is used generically and includes minerals such as hydroxyapatite, dicalcium phosphate, tricalcium phosphate and tetracalcium phos-

10   phate.

We disclose multilayer biocompatible / bioactive functionally graded calcium phosphate in two forms: (i) coatings on variety of substrates, typically stainless steel or titanium, and (ii) self-supporting microspheres. Two types of coatings are depos-

15   ited. The first coating directly on the metal surface is a thin film (0.2-0.5 μm) of dense, highly crystalline HA produced through sol-gel technology at elevated temper-atures, about 400C. This coating serves several purposes: (i) to screen the metal surface from the surrounding tissue; (ii) to provide high-adhesion and nucleation surface for the second CPC-HA coating. The second coating is thicker, porous, film

20   (10-1000 μm) of HA processed at room temperature. The goal is to achieve rapid formation of an adhesive apatite layer through dissolution-precipitation mechanism similar to setting of calcium phosphate cements (CPC). By adding drugs with various concentrations into the CPC colloidal suspension, a direct, *in-situ* encapsulation, and subsequent controlled release of therapeutically active agents from the apatite coatings

25   is achieved. The varying degree of crystallinity of the coating and multi-step coat-ing/impregnation techniques is used to control and customize the CPC-HA resorption process, and thus the rate of drug release from the CPC-HA matrix. Interfacial examination (Figure 2a) shows that the CPC-HA coating is in intimate contact with the underlying SG-HA thin layer. The key feature of the present invention is there-

30   fore that the pre-coated thin SG-HA film acts as a template for the nucleation and growth of room-temperature precipitated apatite in the CPC-HA coating. Such mechanism allows a significant interfacial bonding to develop between the thin SG-HA and the thick CPC-HA, as observed.

35   FTIR spectra of the resulting CPC-HA, Figure 2b, suggest disorder of the crystal structure, attributed to poor symmetry of PO_4 tetrahedra. Effectively, the resulting CPC-HA is amorphous, or very fine, poorly crystalline, non-stoichiometric,

- 15 -

easily resorbable apatite similar to that found in bone mineral, i.e. $Ca_9(PO_4)_{5-x-y}(CO_3)_y(HPO_4)_x(OH)_{1-y/3}$. This CPC-HA had a porosity of about 45% and an average pore size of about 16 nm. The nanopores in the CPC-HA physically immobilize encapsulated biomolecules of similar dimension, such that dissolution of the CPC-HA

5   matrix is responsible for the release kinetics (rather than simply desorption and leaching, as disclosed in previous art). This offers additional mode of drug / gene release control, e.g. through adjustment of the resorption rate (i.e. adjustment of crystallinity) of the CPC-HA microspheres or coatings.

10   We also disclose here the newly discovered approach to achieve the active CaP microsphere, capable to encapsulate any type of drug or biomolecule which can be dispersed in organic liquids or water. The approach combines spheroidization of calcium phosphate cement (CPC) slurry, followed by incubation to precipitate HA phase, Figure 3, 4. The CPC-HA microspheres have a poorly crystalline calcium-

15   deficient apatitic structure, similar to that of natural (bone) apatite, and identical to that determined for the thick CPC-HA coatings grown on SG-HA substrates. The microstructure (i.e. in particular crystallinity and nano-porosity) of the apatite granules can be tailored by adjusting the concentration of the seeding HA sintered sub-micron powder. The greater amount of the seeds, the finer the resulting microstructure results

20   in the matrix phase. The examples of microstructures of crystalline (a) and amorphous (b) CPC-HA produced in our laboratory through changing seeding and process parameters are shown in Figure 5. The key feature of the present invention is that the microspheres are shaped at room temperature, and CPC-HA nucleated and grown also at room temperature, encapsulating the designated organic material, e.g. drug or

25   protein.

In order to achieve better control of drug release, additional film of BM-HA will be deposited on the surface of the microspheres, as illustrated in Figure 3 (Stage 4), and also Figure 6. We have demonstrated previously that excellent quality BM-

30   HA can be grown through solution-precipitation on hydroxyapatite films. BM-HA can be grown on surface of CPC-HA microspheres for final, long term encapsulation.

<u>EXAMPLES</u>

35   **Example 1: CPC-HA / SG-HA Coatings on Stainless Steel**
         Stainless steel metallic substrates (316L) were coated with a 0.6-0.8 μm thin layer of apatite (SG-HA) using the recently developed sol-gel technique. Specifi-

- 16 -

cally, the sol-gel process for preparing a crystallized hydroxyapatite, comprises: (a) hydrolysing a phosphor precursor (phosphite) in a water based medium; (b) adding a calcium salt precursor to the medium after the phosphite has been hydrolysed to obtain a calcium phosphate gel such as a hydroxyapatite gel; (c) depositing the gel on
5   the substrate through dip coating, and (c) calcining the film to obtain crystallized hydroxyapatite, at 400°C for 20min. An inorganic colloidal slurry containing calcium phosphate precursor $Ca(OH)_2$ and calcium phosphate salt monocalcium phosphate anhydrate, was ball milled in ethanol. The two starting inorganic ingredients had particle size 0.3-2 µm and 0.5-4 µm, respectively. The initial Ca/P ratio in the slurry
10 was kept at 1.5. As dissolution and precipitation are the principal mechanisms for apatite development in such system, 5wt% of submicron, crystalline hydroxyapatite powder was used as seeds for heterogeneous nucleation of CPC-HA. The thin film SG-HA surface-modified sample was dip coated in the ethanol suspension of the precursors. After single dip coating, an approximately 30 µm thick layer of porous
15 precursor powder mixture developed on the substrate due to rapid evaporation of ethanol. Due to colloidal nature of the precursors slurry, the thick film develops sufficient structural integrity (i.e. strength and hardness) to accept the next processing step. In this step, the film is exposed to sodium phosphate water-based solution (0.25 M), which is allowed to soak into the open pores of the film, and then placed in an
20 incubator at 37°C, 100% relative humidity, for 24 h. During incubation, the colloidal precursors react with the phosphate liquid and precipitate HA. Microstructure of the resulting CPC-HA thick film is shown in Fig. 5a. The pull adhesion test performed on the coatings according to ASTM C-633 standard revealed adhesion strength of the thick CPC-HA film to the thin SG-HA film of 6.1±1.2 MPa (test on 6 specimens with
25 ~20 µm thick coating). This bonding strength is greater than the tensile strength of bulk CPC-HA reported in literature, which is generally < 2 MPa. The fracture mode was predominantly at the SG-HA/CPC-HA interface, indicating that the interface is the weakest link region. This CPC-HA had a porosity of about 45% and an average pore size of about 16 nm. Macrograph of the coated sample is shown in Figure 1b.
30 Micrograph of the coating interface is illustrated in Figure 2a, and FTIR spectrum in Figure 2b.

Example 2: CPC-HA Coatings on Stainless Steel
          Stainless steel metallic substrates (316L) free of intermediate SG-HA film
35 were coated with CPC-HA, as described in Example 1. During HA incubation period, the coating spontaneously separated from the substrate, as illustrated in Figure 1a. Obviously, the CPC-HA coating did not bond to the metallic substrate, i.e. bonding

- 17 -

strength was zero MPa. Other properties of the coating, that is phase content and porosity, was similar to that obtained in Example 1.

Example 3: BM-HA / SG-HA Coatings on Stainless Steel

5   Stainless steel metallic substrates (316L) were coated with a 0.6-0.8 µm thin layer of apatite (SG-HA) as described in Example 1. One group of samples was annealed at 400°C for 20min to achieve crystalline SG-HA(C) film and another group at 375°C for 60min to achieve amorphous SG-HA(A) film. These films were used as nucleation site for precipitation of BM-HA film. The SG-HA coated samples were

10   immersed into "simulated body fluid" (SBF) of ionic composition (in units of mmol/l) 142 Na^+, 5.0 K^+, 2.5 Ca^{2+}, 1.5 Mg^{2+}, 103 Cl^-, 25 HCO_3^-, 1.4 HPO_4^{2-}, and 0.5 SO_4^{2-}. The SBF was buffered at pH 7.4 with tris(hydroxymethyl)-aminomethane and HCl. This *in-vitro* static deposition (i.e. the SBF was not renewed during the deposition period) at ~24°C produced good quality, dense 3-5 µm thick BM-HA film deposits on flat SG-

15   HA substrates, as illustrated in Figure 6. The crystalline SG-HA(C) film, Fig. 6b, coated with dense BM-HA, whereas amorphous SG-HA(A) film, Fig. 6a, coated with porous BM-HA. The properties of the underlying SG-HA surface modification film can be used to vary the properties, e.g. porosity, of the nucleated and deposited top BM-HA film.

20

Example 4: CPC-HA / SG-HA Coatings with Encapsulated Drug

Stainless steel metallic substrates (316L) were coated with CPC-HA / SG-HA coatings as described in Example 1. In order to assess the possibility of use of CPC-HA for controlled drug release, amethopterin (Sigma Chemicals, USA) was employed

25   as a model drug, in an amount of 5% based on solid phase content of CPC-HA precursors. The drug was mixed with the colloidal suspension of the precursors, before dip coating was performed. All other procedures, e.g. incubation, were performed as in Example 1. During incubation period, 20µm thick CPC-HA coating precipitated encapsulating the drug molecules within the nanopores of the crystalliz-

30   ing HA. After encapsulation, a drug release study was conducted by immersion of the substrates into 20 ml of phosphate buffer saline (PBS, pH=7.4) at constant ratio of (CPC coating weight)/(volume of PBS) of 1 mg/ml. A reference sample coated with hydrogel film was also tested for drug release kinetics. The hydrogel film was prepared by dipping the CPC-HA layer containing the drug into a polymer solution

35   containing 3% polyvinyl alcohol. After drying, the weight gain of the ~20 mg CPC-HA layer due to the additional hydrogel coating was ~0.5 mg, corresponding to the content of polymer film in the CPC-HA matrix of about 2.5%. The samples of PBS

liquid with released drug were periodically taken out (i.e. entire liquid was emptied) and refilled with the same amount of 20 ml of PBS. The drug concentration in the supernatant was determined via an UV-Visible spectroscopy. Figure 7 illustrates the drug release kinetics for the time period of 3 days, for both types of drug-loaded CPC-
5 HA. Although a burst effect was detected for both coatings over the initial period of about 8 h, a slower release is evident for the sample post-coated with hydrogel. A linear relationship was obtained between the amount of drug released and $(time)^{1/2}$ for the release time greater than 8 h. The sustain release period for 8 to 60 h is well described by Fick's law of diffusion. The release kinetics is modified due to the
10 presence of a post-coated thin hydrogel film, suggesting a decreased dffusivity of the drug molecules. However, the burst effect can offer an advantage in the early period after orthopedic/dental surgeries if anti-inflammatory agents were incorporated into the implant devices to avoid acute or severe inflammatory response.

15 **Example 5: CPC-HA Microspheres**

An inorganic colloida slurry containing $Ca(OH)_2$ and monocalcium phosphate anhydrate, is ball milled in ethanol, at Ca/P ratio=1.5 (this stage of the process is similar to CPC-HA coatings processing, as described in Example 1). As dissolution and precipitation are the principal mechanisms for apatite development in such
20 system, small amount (2 wt%) of crystalline HA powder is added to the slurry as seeds for heterogeneous nucleation of HA. Microstructure, in particular nano-porosity, of the apatite granules is tailored by adjusting concentration of the seeding HA sintered sub-micron powder, in the range 1-10wt%. The greater amount of the seeds, the finer the resulting microstructure of the CPC-HA matrix phase. The slurry is
25 atomized and spray dried to produce spheres due to rapid evaporation of ethanol, as illustrated in Figure 3a. Narrow distribution of the microsphere size can be achieved by varying spray parameters (such as spray pressure; slurry viscosity and concentra-tion), with average granular size achievable in the range 10-1000 μm diameter. Any secondary dopant, e.g. drug material, is entrapped in the pores of the sphere at this
30 processing stage, as in Example 4, and also as schematically illustrated in Figure 3b. The colloidal nature of the precursors sol allows a relatively strong bond to develop within the flash-dried precursor microsphere, Stage 1, Fig. 3b. Subsequently, the microspheres were exposed to sodium phosphate water-based solution (0.25 M) and placed in an incubator at 37°C, 100% relative humidity for 24 h, Stage 2. A poorly
35 crystalline, calcium-deficient apatitic structure develops within the microsphere in this process, similar to that of naturally occurring apatite, as described above for the coatings, Stage 3. A poorly crystalline, calcium-deficient apatitic structure develops

within the microsphere in this process, similar to that of naturally occurring (i.e. bone) apatite. The mineralization process in Stage 3 can be expressed as a result of interaction between $Ca(OH)_2$ and $Ca(HPO_4)_2$ in the film exposed to sodium phosphate solution:

5 $$3Ca(HPO_4)_2 + 6\ Ca(OH)_2 \rightarrow Ca_9(PO_4)_5(HPO_4)(OH)$$
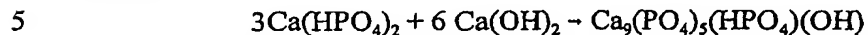
The microstructures of the resulting small (~20 μm) and large (~300 μm) microspheres are presented in Fig. 5. In the final Stage 4 of the process illustrated in Fig. 3b, a thin, dense film of BM-HA may be deposited on surface of the porous HA macrosphere for long-term encapsulation function. To achieve this, HA-CPC spheres

10 with drug encapsulated in its nanopores are immersed into simulated body fluid as described in Example 3.

As will be apparent to those skilled in the art in the light of the foregoing disclosure, many alterations and modifications are possible in the practice of this

15 invention without departing from the spirit or scope thereof. Accordingly, the scope of the invention is to be construed in accordance with the substance defined by the following claims.

WHAT IS CLAIMED IS:

1.　　　　A process for preparing a calcium phosphate phase which comprises:

(a) dispersing a homogenization of monocalcium phosphate $Ca(H_2PO_4)_2$ and calcium hydroxide $Ca(OH)_2$ precursors in a substantially water-free medium to form a paste or viscous slurry of precursors;

(b) drying the precursors slurry ;

(c) admixing a water-based solution of phosphate ions in the slurry to produce a mixture; and

(d) incubating the resulting mixture in a humid environment at a temperature of 20-50°C to dissolve the precursors and precipitate a calcium phosphate phase.

2.　　　　A process as claimed in claim 1 wherein the calcium phosphate phase is hydroxyapatite.

3.　　　　A process as claimed in claim 1 wherein the Ca/P atomic ratio in the precursors mixture is in the range of 1.2 – 1.67 and the mixture has a solids concentration of 30-70 vol%.

.4.　　　　A process as claimed in claim 1 wherein the substantially water-free medium is an alcohol.

5.　　　　A process as claimed in claim 4 wherein the alcohol is ethyl alcohol, methyl alcohol or propyl alcohol

6.　　　　A process as claimed in claim 1 wherein the water-based solution of phosphate ions is sodium phosphate and the humid environment is 50% to 100% Relative Humidity.

7.　　　　A process as claimed in claim 1 wherein the precursors slurry is dried in a prescribed shape.

8.　　　　A process as claimed in claim 7 wherein the shape is a pellet, a coating, a microsphere, or a dental cavity or bone cavity fill.

9.   A process as claimed in claim 1 wherein a crystalline hydroxyapatite powder is added to the precursors slurry to promote crystallization of hydroxyapatite calcium phosphate phase during the incubation period.

10.   A process as claimed in claim 1 wherein a therapeutically active material is added to the precursors slurry for encapsulation during crystallization of the hydroxyapatite calcium phosphate phase during the incubation period.

11.   A process as claimed in claim 10 wherein the therapeutically active material is a drug, a protein, a gene or DNA.

12.   A process as claimed in claim 1 wherein the slurry of precursors is deposited and dried on the surface of a calcium phosphate substrate.

13.   A process as claimed in claim 12 wherein the calcium phosphate substrate is a thin film of hydroxyapatite coating deposited on a metallic substrate using sol-gel process

14.   A process as claimed in claim 1 wherein the precipitated calcium phosphate phase is exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-} ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium phosphate phase.

15.   A process as claimed in claim 10 wherein the precipitated calcium phosphate phase is exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-} ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium phosphate phase.

16.   A process as claimed in claim 1 wherein the slurry of precursors is atomized and spray-dried to obtain microspheres of the precursors.

17.   A process as claimed in claim 10 wherein the slurry of precursors is atomized and spray-dried to obtain microspheres of the precursors.

18.   A process as claimed in claim 16 wherein the microspheres of the precursors are exposed to a water-based solution of phosphate ions and incubated in a

humid environment at a temperature of 20-50°C to promote dissolution of the precursors and precipitation of calcium phosphate phase.

19.   A process as claimed in claim 17 wherein the microspheres of the
precursors are exposed to a water-based solution of phosphate ions and incubated in a humid environment at a temperature of 20-50°C to promote dissolution of the precursors and precipitation of calcium phosphate phase.

20.   A process as claimed in claims 16 wherein the microsphere containing
the precipitated calcium phosphate phase is exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-} ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium phosphate phase.

21.   A process as claimed in claims 18 wherein the microsphere containing
the precipitated calcium phosphate phase is exposed to a solution supersaturated with Ca^{2+} and PO_4^{3-} ions to provide a coating of biomimetic hydroxyapatite film on the precipitated calcium phosphate phase.

22.   A process as claimed in claim 1 wherein the precipitated calcium
phosphate phase is exposed to a solution polymer to form a polymer film on the microsphere surface.

23.   A process as claimed in claim 10 wherein the precipitated calcium
phosphate phase is exposed to a solution polymer to form a polymer film on the microsphere surface.

24.   A process as claimed in claim 16 wherein the precipitated calcium
phosphate phase is exposed to a solution polymer to form a polymer film on the microsphere surface.

25.   A process as claimed in claim 18 wherein the precipitated calcium
phosphate phase is exposed to a solution polymer to form a polymer film on the microsphere surface.

26.   A process as claimed in claim 22 wherein the polymer is a biodegrad-
able polymer

27.   A process as claimed in claim 22 wherein the polymer is polyvinyl
alcohol.

28.   A calcium phosphate phase prepared according to the process of
5   claim 1.

29.   A calcium phosphate phase-substrate prepared according to claim 13.

30.   A microsphere prepared according to the process of claim 16.
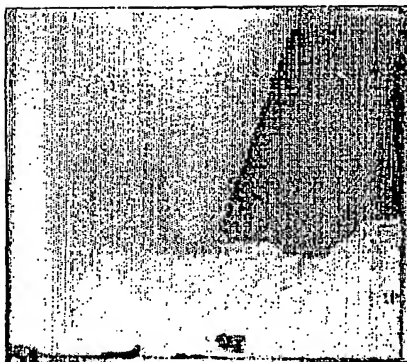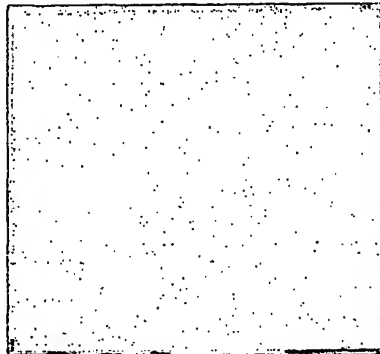
10

1/5
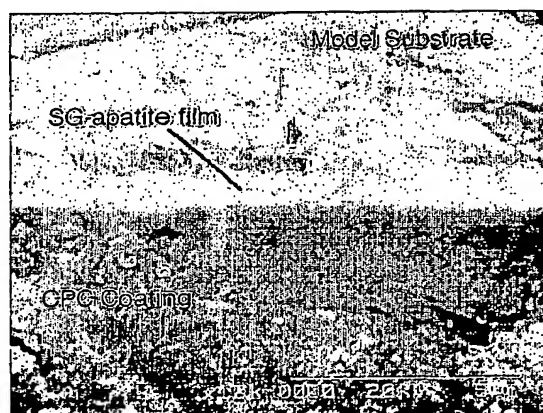

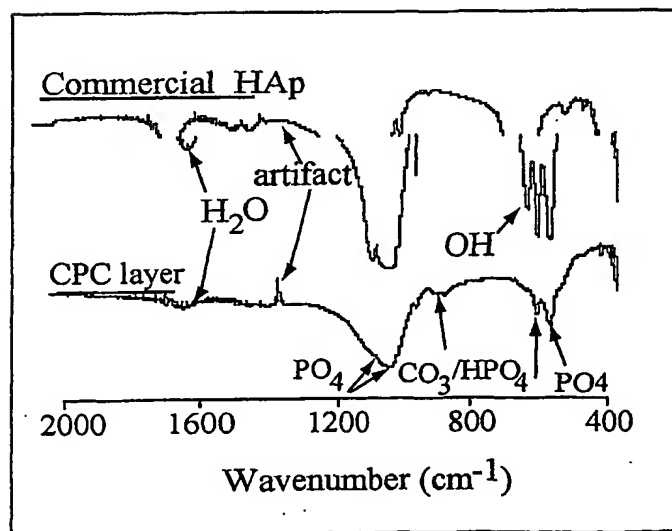FIG. 1a   FIG. 1b

FIG. 2a

FIG. 2b

Spray Gun — Slurry Mix:
CPC powder + liquid = Drug

Warm Air
(~40C)

Microsphere Collector

To Stage 1: microsphere setting (Fig. 3b)

FIG. 3a

Stage 4: Addition of BM-HA encapsulation film

Stage 1: CPC-HA precipitated around encapusulated drug

Stage 2: incubation in phosphate liquid with drug material

Stage 1: CPC-HA Precursors cluster

FIG. 3b

FIG. 4a

FIG. 4b

FIG. 5a

FIG. 5b

FIG. 6a

FIG. 6b

FIG. 7

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61K9/16   A61L27/12   C04B28/34   C01B25/32   A61L27/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   A61K   A61L   C04B   C01B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 17285 A (ETEX CORP) 15 May 1997 (1997-05-15) page 1, line 5 - line 8 page 5, line 23 -page 6, line 4 page 6, line 8 - line 11 page 7, line 4 - line 9 page 10, line 22 - line 27 page 15, line 10 -page 16, line 13 page 16, line 16 - line 28 ---	28
X	DATABASE WPI Section Ch, Week 199110 Derwent Publications Ltd., London, GB; Class D22, AN 1991-068944 XP002213528 & JP 03 016906 A (NIPPON CHEM IND CO LTD), 24 January 1991 (1991-01-24) abstract ---	30

-/--

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2002	30/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	VON EGGELKRAUT, S

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 43558 A (CAP BIOTECHNOLOGY INC ;STEPHAN JAMES E (US); STARLING L BRIAN (US)) 8 October 1998 (1998-10-08) cited in the application page 6, line 3 - line 16	30
A	US 5 958 430 A (SONG LIN ET AL) 28 September 1999 (1999-09-28) cited in the application column 1, line 10 - line 12 column 1, line 55 -column 2, line 36 column 3, line 10 - line 50	13-15
X	column 4, line 51 - line 67	29
A	WO 96 06041 A (NORIAN CORP) 29 February 1996 (1996-02-29) page 3, line 23 -page 4, line 19 page 4, line 25 - line 29 page 6, line 23 -page 7, line 2 page 7, line 18 -page 8, line 14 page 9, line 28 -page 10, line 6 page 10, line 29 -page 11, line 7 page 11, line 11 - line 14 page 11, line 28 -page 13, line 22 claims 1,8,9	1-8,10, 11
A	US 5 525 148 A (CHOW LAURENCE C ET AL) 11 June 1996 (1996-06-11) cited in the application column 1, line 12 - line 18 column 4, line 41 - line 53 column 7, line 1 - line 23 column 11, line 50 - line 65	1,2,10, 11

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9717285	A	15-05-1997	US	5783217 A	21-07-1998
			AU	713435 B2	02-12-1999
			AU	7723596 A	29-05-1997
			CA	2236572 A1	15-05-1997
			EP	0859737 A1	26-08-1998
			JP	2000500110 T	11-01-2000
			NZ	323168 A	29-11-1999
			WO	9717285 A1	15-05-1997
JP 3016906	A	24-01-1991	JP	2756703 B2	25-05-1998
WO 9843558	A	08-10-1998	AU	6946198 A	22-10-1998
			EP	0975285 A1	02-02-2000
			WO	9843558 A1	08-10-1998
			US	6210715 B1	03-04-2001
			US	2001021389 A1	13-09-2001
US 5958430	A	28-09-1999	NONE		
WO 9606041	A	29-02-1996	US	5496399 A	05-03-1996
			AU	3210895 A	14-03-1996
			WO	9606041 A1	29-02-1996
			US	5964932 A	12-10-1999
			US	6053970 A	25-04-2000
			US	5697981 A	16-12-1997
			US	5683496 A	04-11-1997
			US	5846312 A	08-12-1998
US 5525148	A	11-06-1996	AT	210950 T	15-01-2002
			CA	2172538 A1	30-03-1995
			DE	69429526 D1	31-01-2002
			DE	69429526 T2	08-08-2002
			DE	721320 T1	10-04-1997
			EP	0721320 A1	17-07-1996
			ES	2099687 T1	01-06-1997
			GR	97300020 T1	30-07-1997
			JP	10504467 T	06-05-1998
			US	5997624 A	07-12-1999
			WO	9508319 A1	30-03-1995
			US	5954867 A	21-09-1999
			US	5976234 A	02-11-1999

Form PCT/ISA/210 (patent family annex) (July 1992)